0021-7557/09/85-05/421
Jornal de Pediatria

New susceptibility breakpoints in antimicrobial resistance rates of invasive pneumococcal strains

Paula Carolina Bejo Wolkers,[1] Orlando Cesar Mantese,[2] Alan de Paula,[3] Vivieni Vieira Prado Almeida,[4] Paula Augusta Dias Fogaça de Aguiar,[5] Jackelline Rodrigues Alvares,[6] Samanta Cristine Grassi Almeida,[7] Maria Cristina de Cunto Brandileone[8]

Abstract

Objective: To evaluate the impact of new penicillin susceptibility breakpoints on resistance rates of pneumococcal strains collected from children with pneumonia.

Methods: Pneumococcal strains collected from patients admitted with pneumonia were isolated at the clinical analysis lab of Hospital de Clínicas de Uberlândia, Uberlândia, Brazil, and sent to Instituto Adolfo Lutz, São Paulo, Brazil, for further identification, serotyping and determination of antimicrobial susceptibility.

Results: From April 1999 to December 2008, 330 strains of pneumococcus were sent to Instituto Adolfo Lutz; of these, 195 (59%) were collected from patients with pneumonia. One hundred strains collected from patients ≤ 12 years old were analyzed. The patients' age ranged from 1 to 12.6 years old (with mean age of 2.4 and median of 1.7 years). Forty-seven patients were male. The strains were isolated from blood (42%) and pleural fluid (58%). There were 35 oxacillin-resistant strains: according to the criteria defined by the Clinical and Laboratory Standards Institute (CLSI) in 2007 [minimum inhibitory concentration (MIC) ≤ 0.06 µg/mL for susceptibility (S), 0.12 to 1 µg/mL for intermediate resistance (IR), and ≥ 2 µg/mL for full resistance (FR)], 22 strains had IR and 11 strains had FR. According to the current breakpoints defined by the CLSI in 2008 (≤ 2 µg/mL for S, 4 µg/mL for IR and ≥ 8 µg/mL for FR), only one strain had IR to penicillin. There was resistance to co-trimoxazole (80%), tetracycline (21%), erythromycin (13%), clindamycin (13%), and ceftriaxone (one strain simultaneously resistant to penicillin).

Conclusions: When the new breakpoints for in vitro susceptibility were applied, penicillin resistance rates dropped 97%, from 33 to 1%.

J Pediatr (Rio J). 2009;85(5):421-425: Pneumococcus, antimicrobial resistance, pneumonia.

Introduction

Streptococcus pneumoniae, pneumococcus, is one of the most frequent agents of pneumonia, acute otitis media, meningitis, and sinusitis in children.[1,2] It is responsible for high rates of morbidity and mortality among children younger than 5 years old and adults older than 65, especially in developing countries.[2,3] Community pneumonia, one of the most common types of pneumococcal disease in children and adults, usually presents suggestive clinical and radiologic

1. Enfermeira. Aluna, Programa de Pós-Graduação em Ciências da Saúde, Faculdade de Medicina, Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil.
2. Professor, Pediatria, Faculdade de Medicina, UFU, Uberlândia, MG, Brazil. Doutor, Pediatria, Universidade Federal Paulista (UNIFESP), São Paulo, SP, Brazil.
3. Pediatra. Mestre, Ciências da Saúde, Faculdade de Medicina, UFU, Uberlândia, MG, Brazil.
4. Farmacêutica-Bioquímica, Laboratório do Hospital de Clínicas de Uberlândia, Faculdade de Medicina, UFU, Uberlândia, MG, Brazil. Especialista, Microbiologia Clínica, Universidade de São Paulo (USP), São Paulo, SP, Brazil.
5. Médica Patologista Clínica, Laboratório do Hospital de Clínicas de Uberlândia, UFU, Uberlândia, MG, Brazil.
6. Mestre, Ciências, UNIFESP, São Paulo, SP, Brazil. Enfermeira. Pós-graduanda, Programa de Pós-graduação em Ciências da Saúde, Faculdade de Medicina, UFU, Uberlândia, MG, Brazil. Pesquisadora Científica, Instituto Adolfo Lutz (IAL), São Paulo, SP, Brazil.
7. Mestre, Ciências, UNIFESP-EPM. Pesquisadora Científica, IAL, São Paulo, SP, Brazil.
8. Doutora, Ciências, UNIFESP-EPM. Coordenadora, Projeto SIREVA II no Brasil. Seção de Bacteriologia, IAL, São Paulo, SP, Brazil.

This study was conducted at Universidade Federal de Uberlândia (UFU), Uberlândia, MG, Brazil, and at Instituto Adolfo Lutz (IAL), São Paulo, SP, Brazil.

No conflicts of interest declared concerning the publication of this article.

Suggested citation: Wolkers PC, Mantese OC, de Paula A, Almeida VV, de Aguiar PA, Alvares JR, et al. New susceptibility breakpoints in antimicrobial resistance rates of invasive pneumococcal strains. J Pediatr (Rio J). 2009;85(5):421-425.

Manuscript submitted Apr 7 2009, accepted for publication June 17 2009.

doi:10.2223/JPED.1931

signs.[4,5] In patients hospitalized due to pneumococcal pneumonia, bacteremia is detected in approximately 25% of the adults and in 12 to 16% of the children younger than 2 years old.[1,2,5]

The clinical evolution of the pneumococcal infection is influenced by several factors, such as age, underlying disease, topography, severity of infection, and treatment appropriateness.[2] The treatment of pneumococcal infections is based on initial antibiotic therapy, which is usually empirical in terms of etiology and *in vitro* susceptibility to antimicrobial agents. After performing bacterial culture isolation and antibiogram test, the antibiotic therapy can be adequately adjusted. Penicillin is the drug of choice for many pneumococcal diseases[1,2]; however, with the increasing description of penicillin-resistant strains since the 1980s, some alternative regimens have been suggested.[6-9]

Based on the positive response to the treatment with β-lactam antibiotics (penicillin or ampicillin) of patients with invasive pneumococcal diseases (except for meningitis), even when caused by strains with minimum inhibitory concentration (MIC) up to 2.0 µg/mL, a redefinition of the susceptibility categories has been recommended.[6]

With the purpose of monitoring the pattern of resistance to antimicrobial agents and the profile of pneumococcal serotypes in Latin America, the Pan American Health Organization (PAHO) created the Regional System for Vaccines (Sistema Regional de Vacinas, SIREVA) in 1993 with the participation of six countries, including Brazil. Since 1994, this program has been broadened to include other bacteria (*Haemophilus influenzae* and *Neisseria meningitidis* in addition to pneumococcus) and other countries (currently 20 countries take part in this project), being called SIREVA II.[10-12] It is a laboratory surveillance program that analyzes (counts and describes) laboratory characteristics, such as serotype and *in vitro* resistance pattern of the strains collected. Since April 1999, Uberlândia, Brazil, has been participating in the national network of the SIREVA Project by sending pneumococcal strains isolated at the clinical analysis lab of Hospital de Clínicas of Universidade Federal de Uberlândia (HC-UFU), Uberlândia, to Instituto Adolfo Lutz (IAL), São Paulo, Brazil.

As the resistance pattern of antibiotics and the prevalence of pneumococcal serotypes vary according to different populations from different geographic regions and, probably, throughout time as well, it is extremely important to define the profile of the most prevalent serotypes in a given community and to establish the resistance rates to support the choice of the best initial empirical treatment.[13]

Objectives

To assess resistance rates to penicillin and other antimicrobial agents in pneumococcal strains collected from children hospitalized at HC-UFU with diagnosis of pneumonia according to the traditional criteria,[14] as well as penicillin resistance rates based on the current criteria,[15] and the impact of the new criteria on penicillin resistance rates.

Methods

This is a prospective, case series, laboratory surveillance study, whose laboratory data on serotyping and *in vitro* susceptibility to antimicrobial agents of pneumococcal invasive strains were collected from patients hospitalized at HC-UFU with diagnosis of pneumonia. The index case is a pneumococcal strain isolated at the clinical analysis lab of HC-UFU from clinical specimens (blood or pleural fluid) of an inpatient with pneumonia. The patients' individual medical record included identification data (name, birth date, age, sex, address, admission and discharge dates, and length of hospital stay) and clinical data such as medical history, diagnosis, antimicrobial treatment, survival or death, and bacterial source. The medical team providing care to each patient was responsible for the diagnosis and clinical management, without any interference from the laboratory surveillance. Aseptically collected specimens were appropriately processed and seeded in blood culture flasks (blood sample) or chocolate agar and blood agar plates (pleural fluid) within the shortest time possible after collection and immediately after being delivered at the laboratory. Only one pneumococcal isolate per patient was considered in the present study. Pneumococcal strains were isolated and identified at HC-UFU according to internationally described methods and sent to the Bacteriology Department of IAL for specimen confirmation, serotyping and definition of *in vitro* susceptibility to antimicrobial agents.[16,17] Pneumococcal strains were freeze-dried in skim milk at 20% and adequately catalogued.

Antimicrobial susceptibility was assessed using the disc diffusion technique for oxacillin (1 µg), tetracycline, ofloxacin, chloramphenicol, erythromycin, sulfamethoxazole-trimethoprim (co-trimoxazole), vancomycin, and clindamycin in Mueller-Hinton agar plates supplemented with sheep blood at 5% according to the standardized technique.[18,19] Oxacillin-resistant strains (inhibition halo ≤ 19 mm) were submitted to MIC definition for penicillin using broth microdilution and classified as susceptible (S) (MIC ≤ 0.06 µg/mL), intermediate resistant (IR) (MIC = 0.12 to 1 µg/mL), and full resistant (FR) (MIC ≥ 2 µg/mL), according to the traditional criteria.[14,19] According to the current criteria,[15] values for interpretation of MIC results were ≤ 2 µg/mL for S, 4 µg/mL for IR, and ≥ 8 µg/mL for FR. Oxacillin-resistant strains were submitted to ceftriaxone MIC definition and considered S when MIC was ≤ 1µg/mL, IR with MIC = 2µg/mL, and FR with MIC ≥ 4µg/mL.[20] Oxacillin-susceptible strains (inhibition zone > 19 mm) were considered penicillin-susceptible and were not submitted to MIC definition according to the 2007 CLSI standard.

Data were collected from April 1999 to December 2008, and the results were analyzed according to different criteria[14,15] and underwent statistical analysis. Proportions of the antimicrobial susceptibility pattern in different age groups were compared using the chi-square test performed with the Statistical Package for the Social Sciences (SPSS) 8.0 for Windows. Whenever necessary, samples underwent normality and homogeneity tests. Significance level of the null hypothesis was set at 5% ($p < 0.05$).

This study was approved by the Research Ethics Committee of UFU.

Results

During a period of 9 years and 8 moths, from April 1999 to December 2008, 330 samples were sent to the IAL lab. Of these, 195 (59%) were collected from patients with diagnosis of pneumonia. Twenty of these samples were excluded because the specimens were dead when they were delivered to the lab (15) or did not have appropriate records (5). Of the remaining 175 samples, 100 were collected from patients aged ≤ 12 years and were analyzed; 47 (47%) were collected from male individuals. The patients' age ranged from 1 and 12.6 years (mean age = 2.4 years, standard deviation = 2.3 years, and median = 1.7 years) with an interquartile range (IR25-75%) from 1 to 3 years. The number of isolates according to age group was 60 in children up to 2 years old, 32 in children from 2 to 5 years old, and eight among patients between 5 and 12 years old. The number of valid samples collected during each year from 1999 to 2008 was 14, 20, 8, 12, 19, 5, 6, 6, 6, and 4, respectively. The sources of the strains were blood for 42 samples (42%) and pleural fluid for the remaining 58 (58%).

Thirty-five oxacillin-resistant strains (35%) were detected, and according to the traditional criteria,[14,19] 22 (22%) strains were IR to penicillin and 11 (11%) strains were FR (total of 33%). According to the current criteria,[15] only one strain was IR to penicillin (Table 1).

Table 1 - Penicillin-resistance rates according to the 2007 CLSI and 2008 CLSI standards in pneumococcal strains collected from children hospitalized with pneumonia (1999 to 2008)

Resistance	n*	%	n†	%
Intermediate	22	22	1	1
Full	11	11	0	0
Total‡	33	33	1	1

CLSI = Clinical and Laboratory Standards Institute.
* According to the 2007 CLSI standard.
† According to the CLSI 2008 standard.
‡ Total of 100 strains analyzed.

The distribution of the penicillin-resistant strains (only one, collected from a child aged 53 moths) did not show a significant difference according to age group (younger or older than 5 years old).

Reduced susceptibility to co-trimoxazole was detected in 80% of the strains tested (IR = 5 and FR = 75). Resistance to erythromycin and clindamycin was found in 13% of the strains, all of them simultaneously resistant to both antibiotics. Tetracycline resistance rate was 21%, and there was not resistance to chloramphenicol, ofloxacin, rifampicin, and vancomycin.

IR to ceftriaxone was detected in only one strain simultaneously resistant to penicillin.

Discussion

Penicillin resistance was detected in 33% of the strains analyzed – according to the traditional criterion of *in vitro* susceptibility,[14,19] a higher rate than those of 25.9 and 25.6% found in both national[10] and international[3] studies that used the same criterion, respectively. The Brazilian study assessed 4,169 pneumococcal invasive strains, and 878 of these were collected from patients with pneumonia from 2000 to 2005.[10] The surveillance system of the SIREVA project is based on volunteer participation and it has attracted the collaboration of several research centers from different Brazilian states and Latin American countries in the last 13 years.[10-13,16,17,21-24] The distribution of results according to the geographical region has revealed heterogeneity regarding the findings in the Brazilian territory. For instance, among the 94 strains analyzed in the state of Minas Gerais from 1993 to 1998, 12.8% were IR and 2.1% were FR.[17] When this study was enlarged, including samples collected up to 1999 from children up to 6 years old, the total resistance rate was 20.7%,[13] a rate similar to the 20% value found in other local studies carried out in Brazil.[25,26] In the state of Minas Gerais, local studies have revealed rates of 11.8% of oxacillin-resistant strains[27] and 15.5% of penicillin-resistant pneumococcal invasive strains according to the traditional criteria.[28]

According to the traditional criteria, the classification as resistant is clinically important in cases of acute otitis media and meningitis, but it is not relevant in cases of pneumonia.[6,8] In fact, studies involving children and adults with pneumonia caused by penicillin-resistant pneumococcal strains (MIC up to 2 μg/mL) or ceftriaxone-resistant pneumococcal strains (MIC up to 1 μg/mL) have demonstrated an effective response to the treatment with penicillin or third-generation cephalosporin.[6,8] Pharmacokinetic and pharmacodynamic data have confirmed these findings. The presence of serum or tissue concentration of β-lactam antibiotics higher than the MIC for pneumococcus during at least 40 to 50% of the time interval between the doses is a predictive indicator of

clinical effectiveness.[6] Such objective is reached by using penicillin at doses of 8 to 15 million units per day (intervals from 4 to 6 hours) in adults and from 100,000 to 300,000 U/kg/day (4 to 6 doses) in children for the treatment of pneumonia caused by a pneumococcal strain with MIC ≤ 4 µg/mL.[6,29]

New breakpoints for *in vitro* susceptibility to penicillin[15] have been recently suggested. After adopting the new criteria, there was a decrease in the resistance rates of 97%, from 33 to 1%, in the present study. In Brazilian studies, it has been also possible to find a decrease in the penicillin-resistance rates when using different criteria.[11,12] When analyzing 768 invasive strains collected during 2006, among the 168 strains from patients with pneumonia, the authors found a penicillin-resistance rate of 39.3% (IR = 21.4% and FR = 17.9%).[11] When analyzing 874 pneumococcal invasive strains collected during 2007, among the 162 strains from patients with pneumonia, the authors found a penicillin resistance rate of 12.3% (IR) according to the current criterion.[15] In spite of the fact that these results are related to different samples, one collection from 2006[11] and one from 2007,[12] the decrease in the resistance rate was significant (68.7%) for such a short period of time; therefore, we may assume that the main variable was the change in the criterion used to define resistance.

Regarding to the susceptibility of strains to other antimicrobial agents, it possible to conclude that the high resistance rate found for co-trimoxazole (80%) is in agreement with the rates reported in other Brazilian studies (78.5,[17] 65.7,[25] 54.5,[26] and 59.1%), and such high value may compromise the indication of this chemotherapy drug for the treatment of pneumococcal infections. Resistance rates for erythromycin (13%) and clindamycin (13%) remained relatively low; however, these rates are higher than those reported in other Brazilian studies for: erythromycin (2.4,[17] 4.8,[10,11] 5.7,[23] and 3.8%[26]) and clindamycin (2.9[25] and 3.1[26]).

The fact that all 13 erythromycin-resistant strains were also resistant to clindamycin suggests the manifestation of the MLS_B phenotype, characterized by the resistance to macrolides, lincosamides and streptogramin B.[30] There was not *in vitro* resistance to chloramphenicol, ofloxacin, rifampicin or vancomycin.

The fact that there was only one pneumococcal strain IR to ceftriaxone results in an estimated resistance rate of 0.9%, which is lower than the rate found in population surveillance studies conducted in Brazil.[11,12] Of the 412 invasive strains collected from patients without meningitis during 2006[11] and 2007,[12] there was a ceftriaxone resistance rate of 9% (37/412), which was divided into 8.7% of IR (36/412) and 0.3% of FR (1/412). In the USA, the Active Bacterial Core Surveillance (ABCs) Report detected, among 3,514 invasive strains collected during 2007, a cefotaxime resistance rate of 6.9% (IR = 5.5 and FR = 1.4%).[3]

The initial treatment for most pneumococcal infections remains being empirical in terms of etiology and susceptibility to drugs. After the detection of the etiological agent, the choice of the antibiotic is influenced by the laboratory classification (as susceptible or resistant, according to the MIC), and the adoption of new breakpoints must increase the rates of strains reported as being susceptible and consolidate the importance of the use of penicillin for the treatment of pneumococcal pneumonia.[29]

References

1. American Academy of Pediatrics. Pneumococcal infections. In: RED BOOK - Report of the Committee on Infectious Diseases. 27th ed. Illinois: Elk Grove Village; 2006. p.525-37.
2. Fedson DS, Musher MM. Pneumococcal vaccine. In: Plotkin SA, Mortimer EA Jr., editors. Vaccines. 2ed. Philadelphia, PA: W B Saunders; 1994. p.517-64.
3. Centers for Disease Control and Prevention. Active Bacterial Core Surveillance Report, Emerging Infections Program Network. Streptococcus pneumonia, 2007. http://www.cdc.gov/ncidod/dbmd/abcs/survreports/spneu07.pdf. Access: 23/02/2009.
4. Shann F. The management of pneumonia in children in developing countries. Clin Infect Dis. 1995;21 Suppl 3:S218-25.
5. Bartlett JG, Dowell SF, Mandell LA, File Jr TM, Musher FM, Fine MJ. Practice guidelines for the management of community-acquired pneumonia in adults. Infectious Diseases Society of America. Clin Infect Dis. 2000;31:347-82.
6. Heffelfinger JD, Dowell SF, Jorgensen JH, Klugman KP, Mabry LR, Musher DM, et al. Management of community-acquired pneumonia in the era of pneumococcal resistance: a report from the Drug-Resistant Streptococcus pneumoniae Therapeutic Working Group. Arch Intern Med. 2000;160:1399-408.
7. Appelbaum PC. Epidemiology and in vitro susceptibility of drug-resistant Streptococcus pneumoniae. Pediatr Infect Dis J. 1996;15:932-4.
8. Kaplan SL. Review of antibiotic resistance, antibiotic treatment and prevention of pneumococcal pneumonia. Pediatr Respir Rev. 2004;5 Suppl A:S153-8.
9. Okeke IN, Laxminarayan R, Bhutta ZA, Duse AG, Jenkins P, O'Brien TF, et al. Antimicrobial resistance in developing countries. Part I: recent trends and current status. Lancet Infect Dis. 2005;5:481-93.
10. Organización Panamericana de la Salud. Informe Regional de SIREVA II: datos por país y por grupo de edad sobre las características de los aislamientos de Streptococcus pneumoniae, Haemophilus influenzae y Neisseria meningitidis em processos invasores, 2000-2005. Documentos Técnicos. Tecnologias Esenciales de Salud. THS/EV-2007/002. Washington, DC: OPS; 2007.
11. Organización Panamericana de la Salud. Informe Regional de SIREVA II, 2006: datos por país y por grupo de edad sobre las características de los aislamientos de Streptococcus pneumoniae, Haemophilus influenzae y Neisseria meningitidis em processos invasores. Documentos Técnicos. Tecnologias Esenciales de Salud. THS/EV-2008/001. Washington, DC: OPS; 2008.
12. Organización Panamericana de la Salud. Informe Regional de SIREVA II, 2007: datos por país y por grupo de edad sobre las características de los aislamientos de Streptococcus pneumoniae, Haemophilus influenzae y Neisseria meningitidis em processos invasores. Documentos Técnicos. Tecnologias Esenciales de Salud. THS/EV-2008/003. Washington, DC: OPS; 2008.
13. Di Fabio JL, Castaneda E, Agudelo CI, De La Hoz F, Hortal M, Camou T, et al. Evolution of Streptococcus pneumoniae serotypes and penicillin susceptibility in Latin America, Sireva-Vigia Group, 1993 to 1999. PAHO Sireva-Vigía Study Group. Pan American Health Organization. Pediatr Infect Dis J. 2001;20:959-67.

18. National Committee for Clinical Laboratory Standards. Performance standards for antimicrobial disk susceptibility tests: approved standard. NCCLS Publication M2-A5. Villanova, PA: NCCLS; 1997.

19. Clinical and Laboratory Standards Institute/NCCLS. Performance Standards for Antimicrobial Susceptibility Testing; Fifteenth Informational Supplement. CLSI/NCCLS document M100-S15. Wayne, PA: CLSI; 2005.

20. National Committee for Clinical Laboratory Standards. Supplemental Tables. Performance Standards for Antimicrobial Susceptibility Testing; Twelfth Informational Supplement. NCCLS Publication M100-S12, Vol. 22, No. 1. M2-A7 and M7-A5.Villanova, PA: NCCLS; 2002.

21. Brandileone MC, Vieira VS, Zanella RC, Landgraf IM, Melles CE, Taunay AE, et al. Distribution of serotypes of Streptococcus pneumoniae isolated from invasive infections over a 16-year period in the greater São Paulo area, Brazil. J Clin Microbiol. 1995;33:2789-91.

22. Brandileone MC, Vieira VS, Casagrande ST, Zanella RC, Guerra ML, Brandão AP, et al. Characteristics of isolates Streptococcus pneumoniae from middle aged and elderly adults in Brazil: capsular serotypes and antimicrobial sensitivity with invasive infections. Braz J Infect Dis. 1998;2:90-6.

23. Kertesz DA, Di Fabio JL, de Cunto Brandileone MC, Castaneda E, Echaniz-Aviles G, Heitmann I, et al. Invasive Streptococcus pneumoniae infection in Latin American children: results of the Pan American Health Organization Surveillance Study. Clin Infect Dis. 1998;26:1355-61.

24. Brandileone MC, de Andrade AL, Di Fabio JL, Guerra ML, Austrian R. Appropriateness of a pneumococcal conjugate vaccine in Brazil: potencial impact of age and clinical diagnosis, with emphasis on meningitis. J Infect Dis. 2003;187:1206-12.

25. Nascimento-Carvalho CM, Freitas-Souza LS, Moreno-Carvalho OA, Alves NN, Caldas RM, Barberino MG, et al. Cepas invasivas de pneumococo isoladas de crianças e adolescentes em Salvador. J Pediatr (Rio J). 2003;79:209-14.

26. Koeth LM, Felmingham D, Jacobs MR, Rossi F. Antimicrobial resistance of Streptococcus pneumoniae and Haemophilus influenzae in Sao Paulo, Brazil from 1996 to 2000. Int J Antimicrob Agents. 2004;23:356-61.

27. Bedran MB, Camargos PA, Leocádio Filho G, Bedran RM, Najar HC. Susceptibility of Streptococcus pneumoniae to penicillin in the State of Minas Gerais, Brazil from 1997-2004. Braz J Infect Dis. 2005;9:390-7.

28. Mantese OC, Paula A, Moraes AB, Moreira TA, Guerra ML, Brandileone MC. Prevalência de sorotipos e resistência antimicrobiana de cepas ibnvasivas do Streptococcus pneumoniae. J Pediatr (Rio J). 2003;79:537-42.

29. Cardoso MR, Nascimento-Carvalho CM, Ferrero F, Berezin EN, Ruvinsky R, Camargos PA, et al. Penicillin-resistant pneumococcus and risk of treatment failure in pneumonia. Arch Dis Child. 2008;93:221-5.

30. Hyde TB, Gay KV, Stephens DS, Vugia DJ, Pass M, Johnson S et al. Macrolide resistance among invasive Streptococcus pneumoniae isolates. JAMA, 2001;286:1857-62.

Correspondence:
Orlando Cesar Mantese
Avenida Pará, 1979
CEP 38405-320 - Uberlândia, MG - Brazil
Tel.: +55 (34) 3232.2736
Fax: +55 (34) 3232.2736
E-mail: orlando@ufu.br